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Commentary

Regulatory T Cells in Preeclampsia

Some Answers, More Questions?

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Maternal tolerance to the fetus is fundamental for successful reproduction. Derangement of this process may play a role in the pathogenesis of obstetrical diseases, such as preeclampsia (PE), miscarriages, and fetal growth restriction. The action of regulatory T cells (Tregs) has been proposed among the immunosuppressive mechanisms that maintain maternal-fetal immune tolerance.[1] Tregs are a subset of $CD4^+$ T cells specialized in the suppression of the immune response against self-antigens. Tregs are characterized by the expression of the transcription factor, Foxp3, which is required for their differentiation and function.[2] $Foxp3^+$ Tregs can be generated in the thymus, referred to as naturally occurring Tregs (nTregs), or they can be generated in the periphery as a result of the conversion of naïve $CD4^+$ T cells into $Foxp3^+$ Tregs, referred to as induced Tregs (iTregs) or peripheral Tregs.[3] In this issue of *The American Journal of Pathology*, Hsu et al[4] report an enrichment in iTregs in normal pregnancy but not in PE and argue that this defect in iTregs may be central to the pathogenesis of PE.

PE is characterized by hypertension and proteinuria after 20 weeks of gestation. It complicates 3% to 8% of all pregnancies and is a leading cause of maternal and perinatal morbidity and mortality.[5] Epidemiological data suggest that immunological mechanisms may play a role in the pathogenesis of the disease.[6] Decreased exposure to sperm, such as in the case of intracytoplasmic sperm injection for azoospermia, short duration of sexual cohabitation with the father, barrier contraception, and nonpartner donor insemination have been associated with an increased risk of PE.[7–10] The risk of PE is higher in first pregnancies, and multiparous women pregnant with a new partner have a risk similar to nulliparous women. It is still not clear if this effect is due to the change in paternity per se or to the greater risk associated with an increased interpregnancy interval.[11,12] During normal pregnancy, Tregs are present at the maternal-fetal interface as well as peripheral blood. Most studies,[13–15] but not all,[16] have previously reported that there is decreased numbers of Tregs in peripheral blood in women with PE. Moreover, decidual Tregs have also been reported to be lower in PE when compared with normal pregnancies.[17]

Hsu et al[4] analyzed human decidual and peripheral blood Treg populations in healthy and preeclamptic pregnancies. $CD4^+$ $Helios^-$ $Foxp3^+$ Tregs, but not $CD4^+$ $Helios^+$ $Foxp3^+$ Tregs, were overrepresented in the peripheral blood of healthy pregnant individuals when compared with nonpregnant or preeclamptic patients. Although both healthy and preeclamptic pregnancies presented a higher percentage of $Foxp3^+$ Tregs in the decidua compared with the peripheral blood, decidual $Foxp3^+$ Tregs were enriched in $Helios^-$ cells in normal pregnancies but not in PE. These results suggest an induction of $Helios^-$ $Foxp3^+$ Tregs during pregnancy, both in blood and locally in the decidua or in decidual draining lymph nodes that is impaired in PE.

The association of $Helios^-$ $Foxp3^+$ Treg deficiency with PE is particularly interesting in the context of recent findings in mice. A *Foxp3* locus noncoding DNA sequence, CNS1, that is necessary for the generation of iTregs, but not nTregs,[18] was required for the generation of iTreg cells specific for a paternal model alloantigen during pregnancy. These iTregs accumu-

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lated in the decidua and decidua draining lymph nodes. More important, CNS1-deficient females presented increased embryo resorption rates and defective spiral artery remodeling, a feature also observed in human preeclamptic deciduas, in allogeneic, but not in syngeneic, matings.[19]

Interestingly, to evaluate the cause of this iTreg deficit in PE, the authors studied DC-SIGN$^+$ antigen-presenting cells (APCs). Most Foxp3$^+$ cells were associated with DC-SIGN$^+$ APCs in decidua preparations. The number of Foxp3$^+$ and DC-SIGN$^+$ cells was highly correlated in normal pregnancy samples but not in samples from preeclamptic patients, suggesting an impairment in the DC-SIGN$^+$ APC/Treg interaction in PE. The authors demonstrate that decidual CD14$^+$ DC-SIGN$^+$ APCs from preeclamptic pregnancies induced significantly fewer Foxp3$^+$ cells, most of which were Helios$^-$, than CD14$^+$ DC-SIGN$^+$ APCs from healthy pregnancies in alloproliferation assays. Co-culture of CD4$^+$ CD25$^+$ T cells with decidual CD14$^+$ DC-SIGN$^+$ APCs from healthy pregnant women resulted in more Foxp3$^+$ cells, which were largely Helios$^-$, among the proliferating cells than when the co-cultures were performed with CD14$^+$ DC-SIGN$^-$ decidual APCs. The percentage of Foxp3$^-$ T cells (non-Tregs) inversely correlated with the percentage of Foxp3$^+$ cells generated, indicating that the induced cells were functional Tregs. No differences were observed in the percentage of Foxp3$^+$ cells induced between CD14$^+$ DC-SIGN$^+$ and CD14$^+$ DC-SIGN$^-$ APCs from preeclamptic patients, indicating that the capacity of Foxp3$^+$ Treg induction by CD14$^+$ DC-SIGN$^+$ APCs is affected in PE.

This work has some limitations and implications for future studies. The use of Helios to discriminate Treg subtypes is not optimal. Helios is a member of the Ikaros family of transcription factors and was proposed as a marker of nTregs. However, this has later been challenged because Helios expression has also been shown in both *in vitro*– and *in vivo*–induced murine Foxp3$^+$ iTregs,[20–22] and Helios has been proposed as a marker of T-cell activation in both mice and humans.[22] In this context, CD4$^+$ Helios$^-$ Foxp3$^+$ cells could still represent iTregs, albeit on further confirmation. The facts that decidual DC-SIGN$^+$ APCs can induce Helios$^-$ Foxp3 cells in *in vitro* assays and that decidual Tregs are associated with decidual DC-SIGN$^+$ APCs in tissue support the idea that decidual Helios$^-$ Foxp3$^+$ Tregs may, in fact, be iTregs. More recently, neuropilin 1 has emerged as a novel marker that differentiates nTregs and iTregs in mice.[23,24] If neuropilin 1 can also distinguish nTregs and iTregs in humans, it would be interesting to confirm if decidual Helios$^-$ Foxp3$^+$ Tregs and Tregs induced by decidual DC-SIGN$^+$ APCs present an iTreg neuropilin 1 expression pattern.

Helios has also been proposed as a marker of T-cell activation and proliferation, with regressed expression under resting conditions.[21,22] In this framework, Helios$^-$ Tregs in the periphery could reflect the presence of iTregs or, as Gottschalk et al[21] speculate, could also represent a subset of iTregs or nTregs that differ in their stimulation history in either temporal proximity to antigen stimulation or the type of APCs to which they have been exposed. Therefore, it would be important to understand the phenotypical and functional difference between CD4$^+$ Helios$^-$ Foxp3$^+$ and CD4$^+$ Helios$^+$ Foxp3$^+$ Tregs and their precise role in pregnancy.

In addition, it still needs to be determined if this deficit in iTregs is the cause or the consequence of PE, because there is no data available from subjects early in pregnancy, before the onset of clinical disease. Also, whether the iTreg deficit is noted in other placentation disorders, such as recurrent miscarriage or isolated intrauterine fetal growth restriction, remains unknown. It would be important to evaluate whether soluble endoglin, a circulating transforming growth factor-β signaling inhibitor that has been linked with the pathogenesis of PE,[25] may be responsible for this iTreg deficit in PE. Furthermore, it would be interesting to evaluate whether the iTreg deficit in PE leads to defective spiral artery remodeling by altering decidual natural killer cell functions.

In summary, the novel findings by Hsu et al[4] propose an impaired induction of iTregs in PE, possibly through impaired function of decidual APCs. The mechanistic defects behind these findings and their functional significance raise multiple exciting questions regarding the role of immune mechanisms in PE and related disorders.

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